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Form PTO 1390 (REV 5-93)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER P32426
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 10/089508
INTERNATIONAL APPLICATION NO. PCT/GB00/03747	INTERNATIONAL FILING DATE 29 September 2000	PRIORITY DATE CLAIMED 01 October 1999	
TITLE OF INVENTION Transgenic Rodent Comprising A Polynucleotide Encoding A Human UCP3 Polypeptide			
APPLICANT(S) FOR DO/EO/US Alejandro ABUIN and John CLAPHAM			

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98; and Form PTO-1449.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☒ Please amend the specification by inserting before the first line the sentence: This is a 371 of International Application PCT/GB00/03747, filed September 29, 2000, which claims benefit from the following GB Application: 9923334.8, filed October 01, 1999.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☒ An Abstract on a separate sheet of paper.
19. ☐ Other items or information:

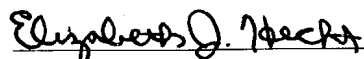
US APPLICATION NO. (if known see 37 CFR 1.50) 10/089508		INTERNATIONAL APPLICATION NO. PCT/GB00/03747		ATTORNEYS DOCKET NO. P32426	
20. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS PTO USE ONLY	
Basic National Fee (37 C.F.R. 1.492(a)(1)-(5)):				890.00	
Search Report has been prepared by the EPO or JPO\$890.00					
International Preliminary Examination Fee paid to USPTO (37 CFR 1.492)\$710.00					
No International Preliminary Examination Fee paid to USPTO (37 CFR 1.492) but international search fee paid to USPTO (37 CFR 1.445(a)(2))\$740.00					
Neither International Preliminary Examination Fee (37 CFR 1.492) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$1,040.00					
International Preliminary Examination Fee paid to USPTO (37 CFR 1.492) and all claims satisfied provisions of PCT Article 33(2)-(4).....\$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$890.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$0.00	
Claims	Number Filed	Number Extra	Rate		
Total claims	16 - 20 =	0	0 x \$18.00	\$0.00	
Independent claims	2 - 3 =	0	0 x \$84.00	\$0.00	
Multiple dependent claims (if applicable)			+ \$280.00	\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$740.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				\$	
SUBTOTAL =				\$740.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)) +				\$	
TOTAL NATIONAL FEE =				\$740.00	
				Amount to be refunded	\$
				charged	\$

- a. ☐ A check in the amount of \$_____ to cover the above fees is enclosed.
- b. ☒ Please charge my Deposit Account No. 19-2570 in the amount of **\$740.00** to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-2570. A duplicate copy of this sheet is enclosed.
- d. ☒ General Authorization to charge any and all fees under 37 CFR 1.16 or 1.17, including petitions for extension of time relating to this application (37 CFR 1.136 (a)(3)).

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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IN THE UNITED STATES INTERNATIONAL EXAMINING AUTHORITY

International Application No.: PCT/GB00/03747
International Filing Date: 29 September 2000
Priority Date Claimed: 1 October 1999
Applicants for DO/US: Alejandro ABUIN and John CLAPHAM
Title of Invention: Transgenic Rodent Comprising A Polynucleotide Encoding A Human UCP3 Polypeptide

Assistant Commissioner for Patents
Box PCT
Washington D.C. 20231

FIRST PRELIMINARY AMENDMENT

Sir:

Preliminary to calculating filing fees and examining this application please amend the application as follows.

In the Specification:

Please add the following paragraph to page 1, directly under the Title of the Invention with the following paragraph:

-- CROSS REFERENCES TO RELATED APPLICATIONS--

This application is a National Stage Application filed under 35 U.S.C. §371 of PCT/GB00/03747, filed on September 29, 2000--.

After page 18, please insert the Abstract that accompanies this Preliminary Amendment.

In the Claims:

2. The transgenic rodent according to claim 1 wherein the polynucleotide encoding a human UCP3 polypeptide is selected from the group consisting of:
 - (a) a polynucleotide comprising a polynucleotide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide sequence of SEQ ID NO:1;
 - (b) a polynucleotide comprising the polynucleotide of SEQ ID NO:1;
 - (c) a polynucleotide having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide of SEQ ID NO:1;
 - (d) the polynucleotide of SEQ ID NO:1;
 - (e) a polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;
 - (f) a polynucleotide comprising a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2;
 - (g) a polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;
 - (h) a polynucleotide encoding the polypeptide of SEQ ID NO:2;
 - (i) a polynucleotide having or comprising a polynucleotide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polynucleotide sequence of SEQ ID NO:1; and
 - (j) a polynucleotide having or comprising a polynucleotide sequence encoding a polypeptide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polypeptide sequence of SEQ ID NO:2.

3. The transgenic rodent according to claim 1 wherein the rodent is selected from the group consisting of:
 - a) mouse; and
 - b) rat.

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4. The transgenic rodent according to claim 1 wherein the polynucleotide encodes a human UCP3 polypeptide of SEQ ID NO:2.
5. The transgenic rodent according to claim 2 wherein the polynucleotide encoding a human UCP3 polypeptide is the polynucleotide of SEQ ID NO:1.
6. The transgenic rodent of claim 1 wherein the human UCP3 polypeptide is expressed predominantly in skeletal muscle.
9. The transgenic rodent of claim 1, wherein said transgenic rodent exhibits reduced body weight.
10. The transgenic rodent of claim 1, wherein said transgenic rodent exhibits increased wound-healing.
11. A method of producing the transgenic rodent as claimed in claim 1, said method comprising the steps of:
 - a) preparing transgene construct comprising coding region of the gene of interest operably linked to an appropriate regulatory sequence;
 - b) removing vector sequences by restriction digest;
 - c) introducing the transgene into the rodent by pronuclear injection; and
 - d) re-transferring the injected eggs into the uteri of pseudo-pregnant recipient mothers.
12. The method of producing the transgenic rodent according to claim 11, wherein the rodent is a mouse and the transgene is introduced into mouse ES cells using a method selected from the group consisting of: electroporation, retroviral vectors, and lipofection for gene transfer.
14. The transgene according to claim 13, wherein the rodent regulatory sequence is the alpha-actin promoter.

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15. A method of determining the phenotypic effect of a compound, said method comprising the steps of:

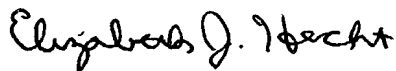
- (1) exposing a transgenic rodent claim 1 to said compound;
- (2) and determining changes in phenotype.

16. The method according to claim 15, wherein the phenotype corresponds to a UCP3-related disease selected from the group consisting of: obesity, diabetes, hyperlipidaemia, body weight disorders, wound healing, cachexia, inflammation, tissue repair, and atherosclerosis.

REMARKS

This Preliminary Amendment is being made upon entry of International Application No. PCT/GB00/03747 into the U.S. National Phase of prosecution. Claims 2-6, 9-12, and 14-16 have been amended to eliminate multiple dependencies and to comply with proper U.S. claim format. Furthermore, attached hereto is a marked-up version of the changes made to the application by the current preliminary amendment. The attached page is captioned, "**Version with markings to show changes made.**"

Respectfully submitted,



Elizabeth J. Hecht
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

CROSS REFERENCES TO RELATED APPLICATIONS

This newly added paragraph to the specification is solely to incorporate continuing application data. No changes have been made. Therefore, a marked up version is not required.

The newly added page to the specification is solely to incorporate the Abstract page. No changes have been made, therefore, a marked up version is not required.

In the claims:

2. [A] The transgenic rodent according to claim 1 wherein the polynucleotide encoding a human UCP3 polypeptide is selected from the group consisting of:
- (a) a polynucleotide comprising a polynucleotide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide sequence of SEQ ID NO:1;
 - (b) a polynucleotide comprising the polynucleotide of SEQ ID NO:1;
 - (c) a polynucleotide having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide of SEQ ID NO:1;
 - (d) the polynucleotide of SEQ ID NO:1;
 - (e) a polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;
 - (f) a polynucleotide comprising a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2;
 - (g) a polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;
 - (h) a polynucleotide encoding the polypeptide of SEQ ID NO:2;

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- (i) a polynucleotide having or comprising a polynucleotide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polynucleotide sequence of SEQ ID NO:1; and
 - (j) a polynucleotide having or comprising a polynucleotide sequence encoding a polypeptide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polypeptide sequence of SEQ ID NO:2.
3. [A] The transgenic rodent according to claim 1 [or 2] wherein the rodent is selected from the group consisting of:
- a) mouse; and
 - b) rat.
4. [A] The transgenic rodent according to [any one of] claim[s] 1 [to 3] wherein the polynucleotide encodes a human UCP3 polypeptide of SEQ ID NO:2.
5. [A] The transgenic rodent according to [any one of] claim[s] 1 to 4] 2 wherein the polynucleotide encoding a human UCP3 polypeptide is the polynucleotide of SEQ ID NO:1.
6. The transgenic rodent of [any one of] claim[s] 1 [to 5] wherein the human UCP3 polypeptide is expressed predominantly in skeletal muscle.
9. The transgenic rodent of [any one of] claim[s] 1, wherein said transgenic rodent [to 8] exhibits[ing] reduced body weight.
10. The transgenic rodent of [any one of] claim[s] 1, wherein said transgenic rodent [to 8] exhibits[ing] increased wound-healing.
11. A method of producing the transgenic rodent [of any one of the preceding] as claimed in claim[s] 1, said method comprising the steps of:

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- a) preparing transgene construct comprising coding region of the gene of interest operably linked to an appropriate regulatory sequence;
- b) removing vector sequences by restriction digest;
- c) introducing the transgene into the rodent by pronuclear injection; and
- d) re-transferring the injected eggs into the uteri of pseudo-pregnant recipient mothers.

12. The [A] method of producing the [a] transgenic rodent according to claim 11, wherein the rodent is a mouse and the transgene is introduced into mouse ES cells[,] using a method selected from the group consisting of: electroporation, retroviral vectors, [or] and lipofection for gene transfer.

14. [A] The transgene according to claim 13, wherein the rodent regulatory sequence is the alpha-actin promoter.

15. A method of determining the phenotypic effect of a compound, said method comprising the steps of:

- (1) exposing a transgenic rodent [of any one of] claim[s] 1 [to 10] to said compound; (2) and determining changes in phenotype.

16. [A] The method according to claim 15, wherein the phenotype [is that of] corresponds to a UCP3-related disease selected from the group consisting of: obesity, diabetes, hyperlipidaemia, body weight disorders, wound healing, cachexia, inflammation, tissue repair, and atherosclerosis.

P32426

Abstract

A transgenic mouse expressing human uncoupling protein 3 (UCP) is disclosed, together with methods of preparation and the uses thereof.

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- (71) Applicant (*for all designated States except US*): SMITHKLINE BEECHAM P.L.C. [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): ABUIN, Alejandro [ES/GB]; SmithKline Beecham Pharmaceuticals, New Frontiers Science Park South, Third Avenue, Harlow, Essex CM19 5AW (GB). CLAPHAM, John [GB/GB]; SmithKline Beecham Pharmaceuticals, New Frontiers Science Park South, Third Avenue, Harlow, Essex CM19 5AW (GB).
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: TRANSGENIC RODENT COMPRISING A POLYNUCLEOTIDE ENCODING A HUMAN UCP3 POLYNUCLEOTIDE

(57) Abstract: A transgenic mouse expressing human uncoupling protein 3 (UCP) is disclosed, together with methods of preparation and the uses thereof.



WO 01/24625 A1

TRANSGENIC RODENT COMPRISING A POLYNUCLEOTIDE ENCODING A HUMAN UCP3 POLYPEPTIDE

The present invention relates to a transgenic animal expressing the human uncoupling protein 3 (UCP3) gene and to the uses thereof. Furthermore the invention relates to methods for constructing the transgenic animal and to the transgenes used in this construction.

With the advent of classical transgenesis and embryonic stem (ES) cell technology in the past two decades, the house mouse (*Mus musculus*) has rapidly become the mammalian model system of choice for the study of gene function. The mouse genome can be manipulated in several ways:

1. Introduction of a "transgene" either randomly (pronuclear injection) or into pre-determined locus (via ES cells).
2. Targeted disruption ("knockout") of the gene of interest.
3. Targeted mutagenesis of the endogenous gene ("knock-in").

1. Introduction of a transgene into the mouse genome.

In general, transgenic mice expressing transgenes represent gain-of-function mutations. Loss-of-function mutations are usually obtained by gene targeting. However, overexpression of a dominant negative mutant protein may result in the functional knockout of the gene of interest (e.g. Stacey et al., Nature 332, 131, 1988). Most commonly, transgenesis is used for the study of tissue- and developmental stage-specific gene regulation, for experiments of the phenotypic effects of transgene expression or for the creation of mouse models of human disease, in particular neurodegenerative conditions like Alzheimer's disease, Huntington's disease, motor neuron disease etc (for general review see R. Jaenisch, Science 240, 1468, 1988). Typically, the transgenic construct is microinjected into the male pronucleus of fertilised eggs (Gordon and Ruddle, Science 214, 1244, 1981), resulting in the random integration into one locus of a varying number of copies, usually in a head to tail array (Costantini and Lacy, Nature 294, 92, 1981). Alternatively, transgenes can be introduced via ES cells, using electroporation, retroviral vectors or lipofection for gene transfer. This has been successfully demonstrated for a number of very large, BAC or YAC derived transgenes (Hodgson et al., Neuron 23, 181, 1999; Lamb et al., Nature Neuroscience 2, 695, 1999). Due to positional effects, expression of a randomly integrated transgene may be inhibited or occur in a non-authentic manner (with respect to the chosen promoter). To overcome these potential problems transgenes can be inserted into pre-determined loci (ROSA26, HPRT etc.) that support transcriptional activity and whose disruption by the insertional mutagenesis is without consequences (Zambrowicz et al., Proc Natl Acad Sci USA 94, 3789, 1997; P. Soriano, Nature Genetics 21, 70, 1999). Again, this technology is ES cell based

and essentially a special case of gene knock-in (see below). Transgenes can also be expressed in other rodents, for example rats (e.g. Breban et al., J Immunol 156, 794, 1996; Garipey et al., J Clin Invest 102, 1092, 1998). In this case the transgene is introduced into the animal by pronuclear injection as the ES cell route is currently only
5 available for mice.

2. Targeted disruption ("knockout") of the gene of interest.

Gene knockout involves the conversion of the gene of interest into a null allele, thus completely disrupting the function of the gene (Joyner AL (editor) Gene targeting: A
10 practical approach. IRL Press, Oxford, England, 1993). Analysis of the resulting phenotype may then allow conclusions as to the function of the gene product. This technology is based on the homologous recombination in embryonic stem cells of a suitable targeting vector with the endogenous gene. Typically, a positive-negative selection strategy is used to enrich for ES cell clones that have undergone the desired
15 recombination event (Thomas and Capecchi, Cell 51, 503, 1987; Soriano et al., Cell 64, 693, 1991). In most cases this results in the replacement of essential coding sequences by foreign DNA (usually a positive selection marker). In a second step the latter is then removed by a site-specific recombinase (Abremski et al., J Biol Chem 261, 391, 1986). Over the last several years tremendous progress has been made to gain spatio-temporal
20 control over the knockout event ("conditional" or "inducible" gene targeting; Rossi and Blau, Curr Opin Biotechnol 9, 451, 1998; A Porter, TIG 14, 73, 1998) but none of the technologies employed has so far yielded entirely satisfactory results. Most avenues toward regulated gene knockout rely on the activity of site-specific recombinases (Cre- or Flp recombinase) that recognize short inverted repeats (LoxP or FRT sites, respectively)
25 and excise the stretch of DNA that is flanked by these repeats (for example "floxed" 5' exon of target gene). Temporal control over recombinase activity (Schwenk et al., Nuc Acids Res 26, 1427, 1998; Mansuy et al., Neuron 21, 257, 1998) thus translates into inducibility of gene targeting (Rossant and McMahon, Genes and Development 13, 142, 1999 (meeting review)). However, a significant disadvantage of recombinase-based
30 approaches is the irreversibility of the knockout.

3. Targeted mutagenesis of the endogenous gene ("knock-in").

Gene knock-in is based on the same principle of homologous recombination as gene knockout. However, a gene knock-in is not designed to ablate the function of the gene but to introduce changes in the coding (or in some instances intron) sequence that are intended to alter the function of the endogenous gene. This may range from a single
5 nucleotide exchange (point mutation; e.g. Cho et al., Science 279, 867, 1998) to the deletion (or addition) of sequences that encode functional protein domains (e.g. Sprengel et al, Cell 92, 279, 1998) or the swapping of the entire mouse coding sequence with, for example, the human cDNA. The latter manipulation is often employed in a pharmaceutical setting, in support of drug development, since some compounds exhibit
10 antagonist or agonist properties against the human but not the murine target.

Mitochondrial uncoupling proteins (UCPs) are inner mitochondrial membrane proteins whose function is to uncouple mitochondrial respiration from ADP
15 phosphorylation (see Ricquier *et al* (1999) J Intern Med 245(6):637-42 for review).

The first member of the family, mitochondrial uncoupling protein 1 (UCP1; Bouillaud et al (1985) Proc Natl Acad Sci 82(2) p445-448; Jacobsson et al (1985) J. Biol. Chem. 260(30) p16250-16254), is expressed exclusively in the brown adipocyte. It functions to uncouple mitochondrial respiration by dissipating the mitochondrial proton
20 gradient, normally used to drive ATP synthesis, to produce heat as a consequence of fatty acid oxidation. In rodents brown adipose tissue contributes to cold adaptation and body weight regulation via non-shivering thermogenesis and diet-induced thermogenesis respectively. However, since little brown adipose tissue (BAT) is present in adult humans, UCP1 is unlikely to play a major role in either of these important homeostatic
25 functions and although many rodentian tissues display a mitochondrial proton leaks that may subserve these functions the precise molecular mechanism by which these leaks occur are not known. The recent discovery of uncoupling protein homologues with wider tissue distribution in both animals and humans may provide some insight into non-shivering and diet-induced thermogenesis in humans.

30 The second member of the uncoupling protein family, uncoupling protein-2, (UCP2) was reported independently by Fleury et al. (Nature Genetics 15, 269, 1997) and Gimeno, et al. (Diabetes 46, 900-906, 1997). UCP2 shares 59% identity to UCP1 at the

amino acid level. However, unlike UCP1, UCP2 is more widely expressed in human tissues predominantly in white adipose tissue, skeletal muscle (a major site of fuel utilisation and thermogenesis) and components of the immune system. The varying level of expression of UCP2 in mouse strains with differential susceptibility to weight gain is
5 consistent with it playing some role in weight gain potential (Fleury et al. 1997 supra). In mice, UCP2 maps close to a quantitative trait locus (QTL) on chromosome 7 associated with obesity. Human UCP2 has been mapped to the homologous region of the long arm of chromosome 11 (Bouchard et al., Human Molecular Genetics 6, 1887-1889, 1997; Solanes et al., J.Biol.Chem 272 25433-25436, 1997).

10 Shortly after the publication of the sequence for UCP2 a third member of the uncoupling protein family was identified and termed UCP3 (WO98/39432 (SmithKline Beecham); Boss et al., FEBS lett 408 39-42, 1997; Vidal-Puig et al., Biochem.Biophys.Res.Comm. 235 79-82, 1997). UCP3 is 73% identical to UCP2 and 59% identical to UCP1 at the amino acid level. In contrast to the wide tissue distribution
15 of UCP2, UCP3 mRNA is predominantly expressed in skeletal muscle. Skeletal muscle is an important site for resting metabolic rate and UCP3 levels in skeletal muscle may be a determinant of energy expenditure and metabolic efficiency in Pima Indians (Schrauwen et al., Diabetes 48 146-149, 1999). UCP3 also maps to 11q13 and is adjacent to UCP2 to within 100 kb (Gong et al., Biochem.Biophys.Res.Comm. 256 27-32, 1997; Solanes et
20 al., 1997 supra) suggesting that they are evolutionarily very close. UCP3 has also been implicated in wound healing (SmithKline Beecham plc patent application WO00/02577).

There is a need to characterise further the UCP genes and the UCP polypeptides expressed therefrom, to determine the function of the polypeptides and to investigate the effect of increased or reduced expression and its relevance to disease. In addition the
25 consequences of altered spatial or temporal expression of the UCP polypeptide need to be investigated as well as the effects of altered UCP polypeptides, where such alterations may have arisen through mutation for example. There is also a need to provide a means to identify and evaluate (with regard efficacy and safety) chemical compounds that modulate the activity of the UCP genes or polypeptides. Such modulation may, for example, be
30 afforded by compounds which bind to and activate (agonist) or inhibit activation (antagonist) of the UCP polypeptide. Compounds identified thereby could be useful in

therapy of diseases where inappropriate expression of the UCP polypeptide or a mutated form of the UCP polypeptide is implicated. Such diseases include, but are not limited to, obesity, diabetes, hyperlipidaemia, body weight disorders, wound healing, cachexia, inflammation, tissue repair and atherosclerosis.

5 One method for facilitating such studies, in particular studies of body weight disorders, diabetes, obesity and inflammation, and the evaluation of compounds that have potential to treat such disorders and diseases, is the generation and use of a transgenic rodent capable of expressing the human UCP3 polypeptide. Transgenic mice of the invention which have been engineered to overexpress human UCP3 are herein shown to
10 have significantly reduced body weight compared to age-matched wild type controls, despite showing increased food intake, thereby confirming their suitability for such studies.

 The present invention provides a transgenic rodent having a genome comprising a
15 polynucleotide encoding a human UCP3 polypeptide under the control of a regulatory sequence facilitating expression of said polypeptide. Such polynucleotides include:
 (a) a polynucleotide comprising a polynucleotide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide sequence of SEQ ID NO:1;
 (b) a polynucleotide comprising the polynucleotide of SEQ ID NO:1;
20 (c) a polynucleotide having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide of SEQ ID NO:1;
 (d) the polynucleotide of SEQ ID NO:1;
 (e) a polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ
25 ID NO:2;
 (f) a polynucleotide comprising a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2;
 (g) a polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ
30 ID NO:2;
 (h) a polynucleotide encoding the polypeptide of SEQ ID NO:2;

(i) a polynucleotide having or comprising a polynucleotide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polynucleotide sequence of SEQ ID NO:1; and

(j) a polynucleotide having or comprising a polynucleotide sequence encoding a polypeptide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polypeptide sequence of SEQ ID NO:2.

In a preferred aspect the transgenic rodent is a mouse or rat, preferably a mouse.

The assembly of a transgenic construct follows standard cloning techniques, that are well known in the art (for example see Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The cDNA to be overexpressed can be prepared from a mRNA extracted from a relevant tissue, preferably a tissue in which the protein of interest is known to be expressed for example skeletal muscle. The cDNA, along with the promoter of choice and other components such as artificial introns and reporter genes, can then be inserted into a cloning vector by restriction digest and ligation. Suitable cloning vectors for the assembly of transgenes provide for acceptable yields of DNA. Vectors such as pBluescript are particularly preferred as in addition to good yield, they provide desirable unique restriction sites flanking the transgene (for example BssHII in pBluescript) for convenient removal of the vector portion of the construct prior to pronucleus injection. Should any of the components of the transgene inserted into the vector contain within their sequence additional restriction sites that are identical to the flanking restriction sites, such restriction sites will clearly not be unique and so alternative restriction sites must be identified or introduced for effective vector removal without transgene disruption.

In a further embodiment the transgene can be isolated from genomic DNA.

The expression of the transgene in the host genome may be controlled temporally and/ or spatially by placing the transgene under the control of an appropriate regulatory sequence, for example a promoter. The choice of promoter depends on the biological question that the mouse model is intended to answer. Most transgenes contain tissue-specific promoters that, in the best case scenario, lead to a spatially and temporally authentic (with respect to the endogenous gene) expression pattern of the transgene. Other promoters provide for ubiquitous expression across the entire organism. One example of

a tissue specific promoters is the alpha-actin promoter that drives transcription in skeletal muscle. There are many other tissue-specific promoters that can be used. Such promoters include, but are not limited to, the calcium-calmodulin dependent Kinase II (CamKII) promoter for expression in neurons and neurosecretory cells; albumin promoter
5 for hepatocytes; insulin promoter for pancreatic beta cells; rhodopsin promoter for retinal rods and cones; myogenin promoter for skeletal muscle; promoters of certain keratins for dermis; etc.

Regulatory sequences, such as promoters, are operably linked to the coding sequence of the gene of interest in a manner that will permit the required temporal and
10 spatial expression of the transgene. Methods of so linking regulatory sequences to cDNAs to facilitate their expression are widely known in the art. Such methods include directly ligating a polynucleotide sequence comprising a regulatory sequence to the coding region of the transgene. Additional polynucleotide sequences may be included that modulate expression in the required manner. Examples of additional sequences
15 include enhancer elements, artificial introns and others. In addition the nucleotide sequence of a known promoter, or other regulatory sequence, may be modified to increase levels of expression. Such modifications can be achieved using, for example, site-directed mutagenesis methods well known in the art (see Sambrook et al, *supra*).

In addition to modifying the sequence of regulatory elements to enhance, or
20 otherwise change, expression levels, the coding sequence of the gene of interest may be modified to enhance or otherwise affect expression levels. For example if the transgene is from a different species than the host, the codon usage of the transgene can be altered to match more closely that of the host. It is well known in the art that different organisms use the 64 coding and stop codons at different frequencies. Codons that are infrequently
25 used in an organism are termed "rare codons". If a transgene includes a codon that is a rare codon in the host, expression levels may be severely reduced. One solution is to replace one or more rare codons in the transgene with codons that are frequently used in the host. Other modifications to the transgene sequence include modifying the polynucleotide sequence surrounding the start codon (the initiator methionine encoding
30 codon) to make this more closely match the consensus "Kozak" sequence (A/G CCATGG, where the ATG in bold is the start codon; see for example Kozak, M., *Nucleic Acids Res* (1984) May 11;12(9):3873-3893)). In the transcribed mRNA molecule the

Kozak sequence is believed to provide the optimal environment for initiation of translation of the polypeptide.

Preferably, prior to the introduction of the transgene into the host cell, the vector portions are removed by restriction enzyme digestion, for example by using restriction
5 sites in the vector that flank the transgene. Thus the genetic material that is actually introduced into the host cell will preferably comprise the coding sequence of the gene of interest and the regulatory sequences to which it has been operably linked together with other potential components of the transgene, for example a reporter gene. More preferably the genetic material will have only the transgene and the regulatory sequences
10 to which it has been operably linked.

There are a number of techniques that permit the introduction of genetic material, such as a transgene, into the rodent germline. The most commonly used protocol comprises direct injection of the transgene into the male pronucleus of the fertilised egg (Hogan et al., *Manipulating the mouse embryo (A laboratory manual)* Second edition,
15 CSHL Press 1994). The injected eggs are then re-transferred into the uteri of pseudo-pregnant recipient mothers. Some of the resulting offspring may have one or several copies of the transgene integrated into their genomes, usually in one integration site. These "founder" animals are then bred to establish transgenic lines and to back-cross into the genetic background of choice. It is convenient to have the transgene insertion on both
20 chromosomes (homozygosity) as this obviates the need for repeated genotyping in the course of routine mouse husbandry.

An alternative method to introduce the transgene into mice is the random insertion into the genome of pluripotent embryonic stem (ES) cells, followed by the production of chimeric mice and subsequent germline transmission. Transgenes of up to
25 several hundred kilobases of rodentian DNA have been used to produce transgenic mice in this manner (for example Choi et al., *Nature Genet.* 4, 117-123 (1993); Strauss et al., *Science* 259, 1904-07 (1993)). The latter approach can be tailored such that the transgene is inserted into a pre-determined locus (non-randomly, for example ROSA26 or HPRT) that supports ubiquitous as well as tissue specific expression of the transgene (Vivian et al., *BioTechniques* 27, 154-162 (1999)).
30

The transgenic rodent is subsequently tested to ensure the required genotypic change has been effected. This can be done by, for example, detecting the presence of the

transgene by PCR with specific primers, or by Southern blotting of tail DNA with a specific probe. Testing for homozygosity of the transgene insertion may be carried out using quantitative Southern blotting to detect a twofold difference in signal strength between hetero- and homozygous transgenic rodents. Once the desired genotype has been confirmed the transgenic rodent line is subjected to various tests to determine the gain-of-function phenotype. The tests involved in this phenotypic characterisation depend on what genotypic change has been effected, and may include, for example, morphological, biochemical and behavioural studies.

In a preferred aspect of the present invention the polynucleotide encoding the UCP3 polypeptide, as defined hereinabove, is predominantly expressed in skeletal muscle. Such near-exclusive expression may be facilitated by the transcriptional activity of skeletal muscle specific promoters. In a preferred embodiment the skeletal muscle-specific promoter is the alpha-actin promoter.

Phenotypic tests can be devised for examining the effect of overexpressing the human UCP3 gene. Such tests are based on the hypothesis that UCP3 protein uncouples the respiratory chain in the mitochondria from the generation of energy rich molecules (NADP, NADPH, and ultimately ATP, GTP), with the result that there is excess heat production. Thus, simple measurements like weight gain, food intake and body temperature are preferred phenotypic tests for the initial analysis of the UCP3 transgenic rodents. Subsequently, glucose clearance and other parameters of glucose homeostasis can be investigated. In addition the time required for wound healing and general behavioural trends may be investigated. Based on the results of these studies, further more specific tests can be devised to give a more detailed analysis of the consequences of UCP3 expression.

Although one function of such transgenic rodents is that of elucidating the function of a gene of interest, they may also be used in the validation of the polypeptide expressed from the transgene as a drug target. The transgenic rodents of the present invention can also be used to test the efficacy of a drug and a drug administration regime for the treatment of UCP3-related diseases such as obesity, diabetes, hyperlipidaemia, body weight disorders, wound healing, cachexia, inflammation, tissue repair and atherosclerosis. Thus in a further aspect the present invention provides a method of determining the

phenotypic effect of a compound comprising exposing a transgenic rodent overexpressing human UCP3 as hereinabove described to said compound and determining changes in phenotype.

Once established, these transgenic rodents could be used to investigate the effects of various drug treatments on the course of the disease (in the animal model setting). In addition, transgenic overexpression models may produce surprising, unexpected results by way of the resulting phenotype. This could result in the identification of "new" disease indications, or serve as a warning, for example when the transgenic rodents display developmental abnormalities or develop tumours.

The following definitions are provided to facilitate understanding of certain terms used frequently hereinbefore.

A "Transgene" comprises a polynucleotide, isolated from nature, which has been manipulated *in-vitro* and subsequently introduced into the genome of the same or a different species in either the native or modified forms, such that it is stably and heritably maintained in that genome. Native forms include unmodified polynucleotides isolated from a source different to that into which it is subsequently introduced, for example a human polynucleotide sequence introduced into a mouse genome. Modified polynucleotides include those which have one or more nucleotide substitutions, deletions, insertions or inversions. Native or modified polynucleotides may be operably linked to a heterologous promoter, or other regulatory sequence, from a different gene within the same species or from a gene in a different species. A polynucleotide is operably linked to a regulatory sequence when, for example, it is placed under the transcriptional control of said regulatory sequence. The polynucleotide may or may not encode a polypeptide, and if a polypeptide is expressed from the polynucleotide, said polypeptide may or may not be full-length relative to that encoded by the original polynucleotide isolated. The term transgene is generally used to refer to the polynucleotide and the regulatory sequences to which it is operably linked.

An organism into which a transgene has been introduced is termed a "transgenic" organism.

"Regulatory sequences" refer to DNA or RNA polynucleotide sequences, which are usually non-coding, that are involved in the regulation of transcriptional activity or

tissue-specific enhancement or silencing of gene transcription. Such regulatory sequences include promoters and enhancers.

- "Identity" as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.
- Preferred parameters for polypeptide sequence comparison include the following:
- 1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)
 - Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA.* 89:10915-10919 (1992)
 - Gap Penalty: 12
 - Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:

- 5 1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

- 10 Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one
 15 nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is
 20 determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the numerical percent of the respective percent identity(divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

- wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides
 25 in SEQ ID NO:1, and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%,etc., and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the
 30 polypeptide encoded by the polynucleotide following such alterations.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group

5 consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference

10 sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y),$$

15 wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

Claims

1. A transgenic rodent having a genome comprising a polynucleotide encoding a human UCP3 polypeptide under the control of a regulatory sequence facilitating expression of said polypeptide.
2. A transgenic rodent according to claim 1 wherein the polynucleotide encoding a human UCP3 polypeptide is selected from the group consisting of:
 - (a) a polynucleotide comprising a polynucleotide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide sequence of SEQ ID NO:1;
 - (b) a polynucleotide comprising the polynucleotide of SEQ ID NO:1;
 - (c) a polynucleotide having at least 95%, 96%, 97%, 98%, or 99% identity to the polynucleotide of SEQ ID NO:1;
 - (d) the polynucleotide of SEQ ID NO:1;
 - (e) a polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;
 - (f) a polynucleotide comprising a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2;
 - (g) a polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 95%, 96%, 97%, 98%, or 99% identity to the polypeptide sequence of SEQ ID NO:2;
 - (h) a polynucleotide encoding the polypeptide of SEQ ID NO:2;
 - (i) a polynucleotide having or comprising a polynucleotide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polynucleotide sequence of SEQ ID NO:1; and
 - (j) a polynucleotide having or comprising a polynucleotide sequence encoding a polypeptide sequence that has an Identity Index of 0.95, 0.96, 0.97, 0.98, or 0.99 compared to the polypeptide sequence of SEQ ID NO:2.

a) mouse; and
b) rat.

5

5. A transgenic rodent according to any one of claims 1 to 4 wherein the polynucleotide encoding a human UCP3 polypeptide is the polynucleotide of SEQ ID NO:1.

10

15

8. The transgenic rodent of claim 7 wherein the skeletal muscle-specific promoter is alpha-actin.

20

10. The transgenic rodent of any one of claims 1 to 8 exhibiting increased wound-healing.

25

- a) preparing transgene construct comprising coding region of the gene of interest operably linked to an appropriate regulatory sequence;
- b) removing vector sequences by restriction digest;
- c) introducing the transgene into the rodent by pronuclear injection; and

30

12. A method of producing a transgenic rodent according to claim 11, wherein the
5 rodent is a mouse and the transgene is introduced into mouse ES cells, using
electroporation, retroviral vectors or lipofection for gene transfer.

10

15. A method of determining the phenotypic effect of a compound comprising exposing a transgenic rodent of any one of claims 1 to 10 to said compound and determining changes in phenotype.

20

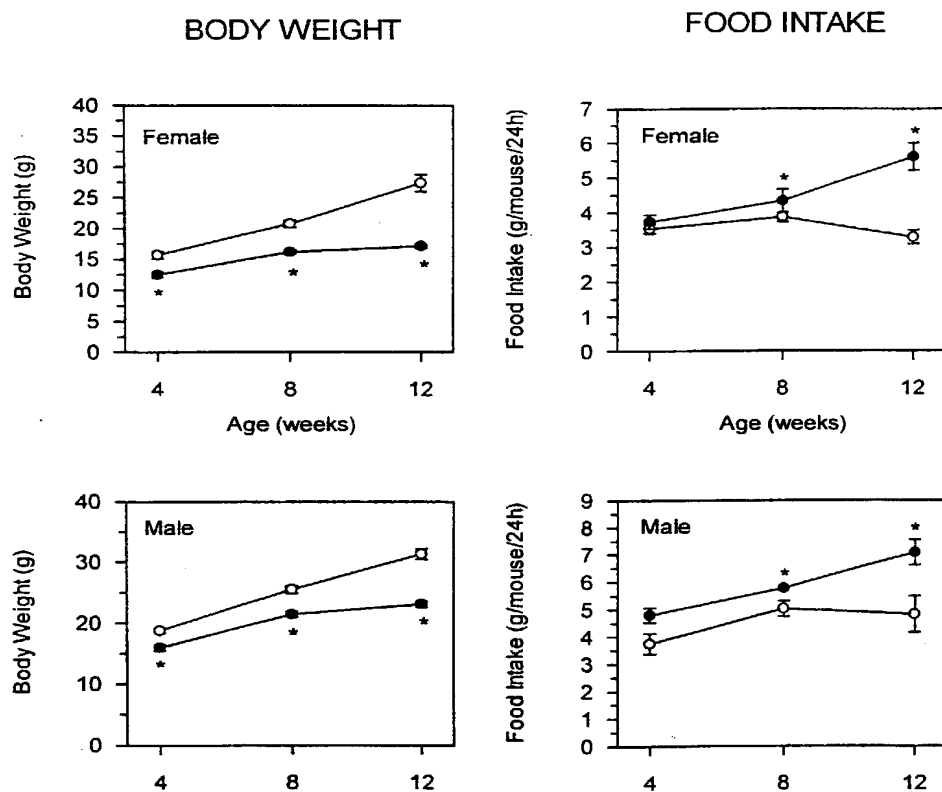


Figure 1

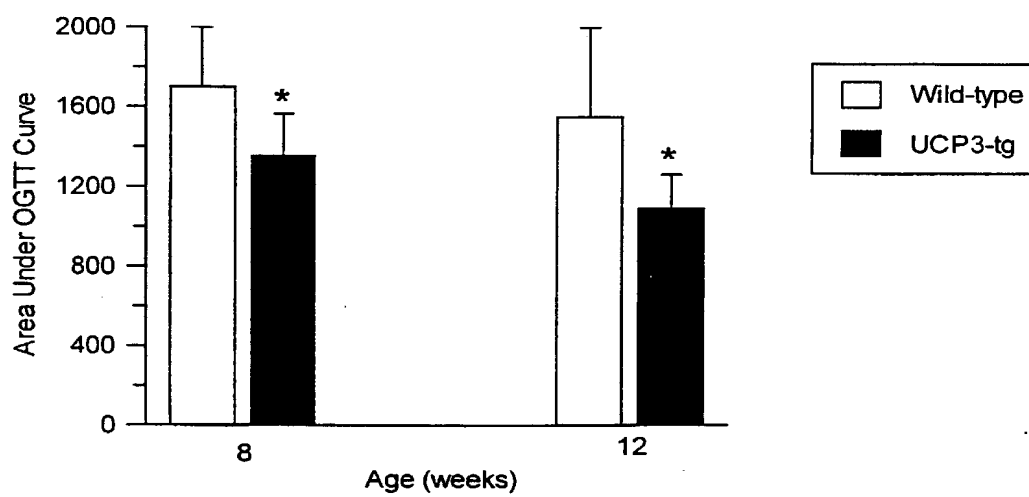


Figure 2

WO 01/24625

PCT/GB00/03747

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<212> PRT
<213> Homo sapiens

5

<400> 2

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20 25 30
10 Pro Leu Asp Thr Ala Lys Val Arg Leu Gln Ile Gln Gly Glu Asn Gln
35 40 45
Ala Val Gln Thr Ala Arg Leu Val Gln Tyr Arg Gly Val Leu Gly Thr
50 55 60
Ile Leu Thr Met Val Arg Thr Glu Gly Pro Cys Ser Pro Tyr Asn Gly
15 65 70 75 80
Leu Val Ala Gly Leu Gln Arg Gln Met Ser Phe Ala Ser Ile Arg Ile
85 90 95
Gly Leu Tyr Asp Ser Val Lys Gln Val Tyr Thr Pro Lys Gly Ala Asp
100 105 110
20 Asn Ser Ser Leu Thr Thr Arg Ile Leu Ala Gly Cys Thr Thr Gly Ala
115 120 125
Met Ala Val Thr Cys Ala Gln Pro Thr Asp Val Val Lys Val Arg Phe
130 135 140
Gln Ala Ser Ile His Leu Gly Pro Ser Arg Ser Asp Arg Lys Tyr Ser
25 145 150 155 160
Gly Thr Met Asp Ala Tyr Arg Thr Ile Ala Arg Glu Glu Gly Val Arg
165 170 175
Gly Leu Trp Lys Gly Thr Leu Pro Asn Ile Met Arg Asn Ala Ile Val
180 185 190
30 Asn Cys Ala Glu Val Val Thr Tyr Asp Ile Leu Lys Glu Lys Leu Leu
195 200 205
Asp Tyr His Leu Leu Thr Asp Asn Phe Pro Cys His Phe Val Ser Ala
210 215 220
Phe Gly Ala Gly Phe Cys Ala Thr Val Val Ala Ser Pro Val Asp Val
35 225 230 235 240
Val Lys Thr Arg Tyr Met Asn Ser Pro Pro Gly Gln Tyr Phe Ser Pro
245 250 255
Leu Asp Cys Met Ile Lys Met Val Ala Gln Glu Gly Pro Thr Ala Phe

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260 265 270
 Tyr Lys Gly Phe Thr Pro Ser Phe Leu Arg Leu Gly Ser Trp Asn Val
 275 280 285
 Val Met Phe Val Thr Tyr Glu Gln Leu Lys Arg Ala Leu Met Lys Val
 5 290 295 300
 Gln Met Leu Arg Glu Ser Pro Phe
 305 310

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TRANSGENIC RODENT COMPRISING A POLYNUCLEOTIDE ENCODING A HUMAN UCP3
POLYNUCLEOTIDE

the specification of which (check one)

☐ is attached hereto.

☒ was filed on 29 September 2000 as Serial No. PCT/GB00/03747
and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or Inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Number	Country	Filing Date	Priority Claimed
9923334.8	GB	01 October 1999	Yes

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

Application Number	Filing Date
--------------------	-------------

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Serial No.	Filing Date	Status
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I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

Customer Number 20462.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

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